The thesis entitled ***"Enantioselective Synthesis of (–)-8,4′-Oxyneolignans & Synthesis and Bioevaluation of Nitrogen Containing Heterocyclic Compounds"*** has been divided into two parts, Part A and Part B, and each part is further divided into chapters. Each chapter except chapter-I (Introduction), is further sub-divided into the following sections: Introduction, Present work, Results and discussion, Experimental section, Conclusions, References and Spectroscopic data.

PART A: ***Deals with the Enantioselective Synthesis of (–)-8,4′-Oxyneolignans, and is divided into Chapter I and Chapter II.***

Chapter I: Introduction

This chapter deals with the introduction of important plant metabolites viz., lignans, neolignans and 8,4′-oxyneolignans and summarizes some of the reported approaches towards the (–)-8,4′-oxyneolignans. This chapter also deals with the introduction of the glycolate Evans aldol reaction and our current approach of its application in the enantioselective synthesis of (–)-8,4′-oxyneolignans.

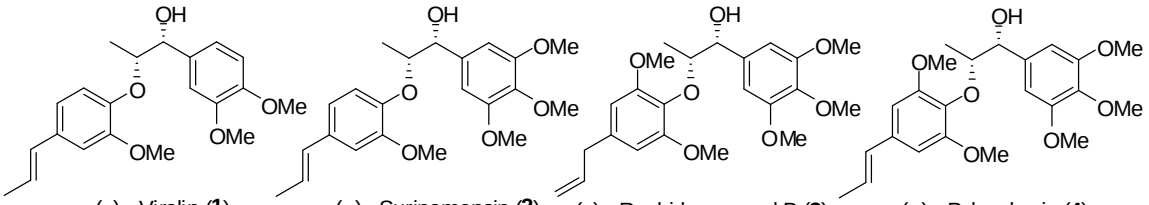


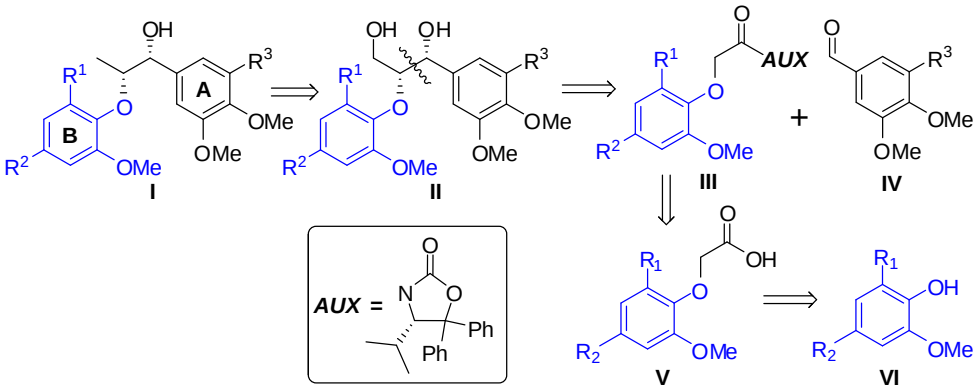
Figure 1: Srtuctures of (–)-virolin (**1**), (–)-surinamensin (**2**), (–)-raphidecursinol B (**3**) and (–)-polysphorin (**4**).

Chapter-II: Enantioselective Synthesis of (–)-8, 4′-Oxyneolignans

This chapter details our efforts in the enantioselective synthesis of four biologically active 8,4′-oxyneolignans., (–)-Virolin, (–)-Surinamensin, (–)-Raphidecursinol B and (–)-Polysphorin.

(–)-Virolin, (–)-surinamensin, (–)-raphidecursinol B and (–)-polysphorin are optically active 8,4′-oxyneolignans, found in various medicinally important natural

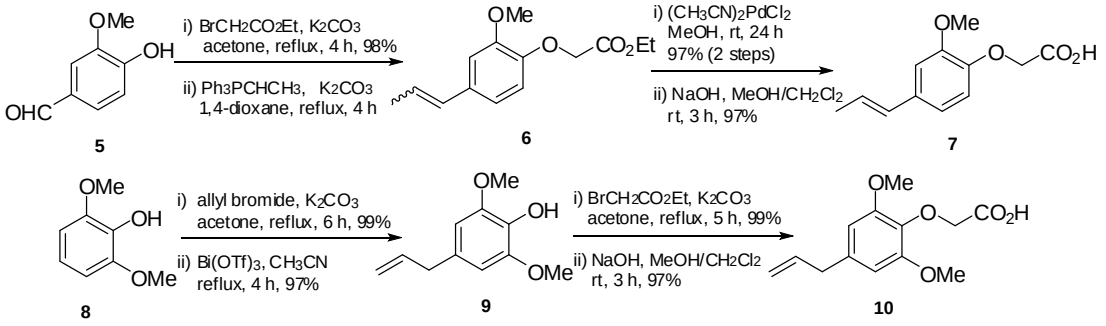
products. These compounds, isolated from *Myristicaceae* and other primitive plant families in neotropical regions, belong to one of the major class of neolignans containing a chiral aryloxy C(8)-*O*-C(4′) ether linkage. Virolin (**1**) and surinamensin (**2**), isolated from the leaves of *Virola surinamensis (Myristicaceae)*, show activity against leishmaniasis, fungal and other vector-brone diseases. Raphidecursinol B (**3**) isolated from the seeds (nut meg) of *Myristica Fragrans Houtt (Myristicaceae)* exhibit activity against plasmodium falciparum. Polysphorin (**4**) isolated from *piper polysphorum C* in China and also from *Rhaphidopora decursiva* in Vietnam, display anti-malarial activity. The four biologically active 8,4′-Oxyneolignans - (–)-virolin (**1**), (–)-surinamensin (**2**), (–)-raphidecursinol B (**3**) and (–)-polysphorin (**4**) - were synthesized using glycolate *Evans syn-aldol reaction* as key step.



Scheme 1: Retro-synthetic strategy and the building blocks

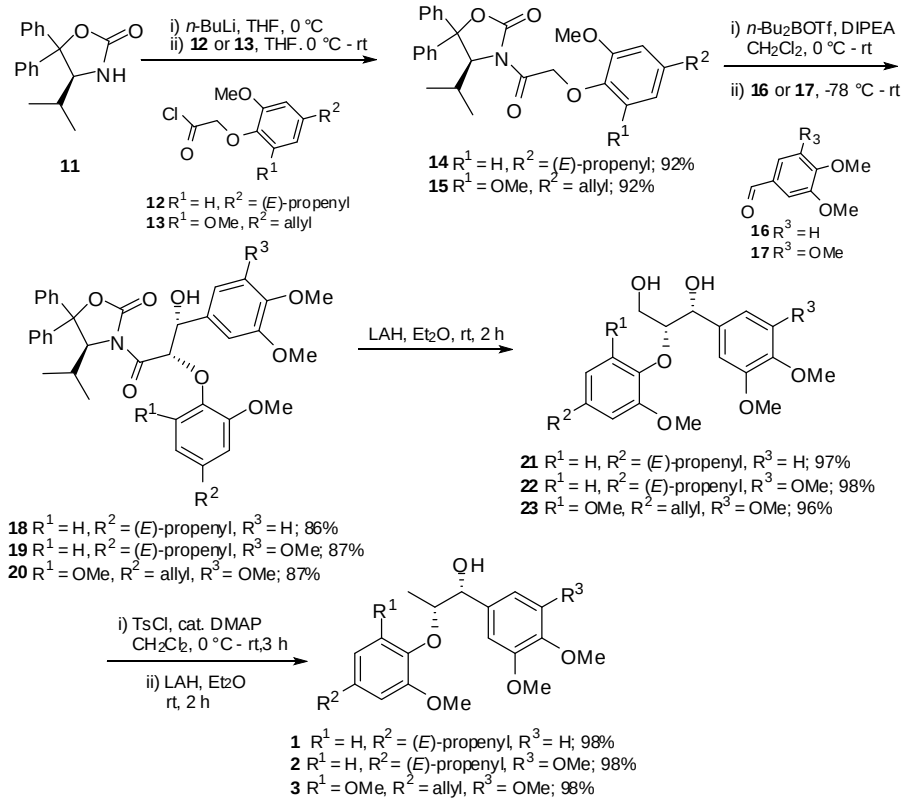
Our strategy is depicted retrosynthetically in Scheme 1. It was envisaged that the reductive deoxygenation of the primary hydroxyl group of the diol (**II**) resulting from the auxiliary assisted aldol reaction of suitable substrates would afford the 1-aryl-2-aryloxypropanol (**I**) moiety in the required stereochemistry. The auxiliary linked chiral precursor (**III**) could easily be generated from aryloxyacetic acid (**V**), which in turn could be readily prepared from commercially available starting materials (**VI**).

The aryloxyacetic acid derivative (**7**), A-ring precursor for virolin and surinamensin was assembled starting from vanillin (**5**). The aryloxyacetic acid derivative (**10**), A-ring building block of raphidecursinol B and polysphorin was generated starting from 2,6-dimethoxyphenol (**8**) (Scheme 2).



Scheme 2: Preparation of aryloxy acetic acid derivatives **7** & **10**

With both A- and B-ring counterparts at our disposal, we proceeded to the crucial auxiliary assisted aldol reaction. Reaction of lithium salt of the auxiliary (**11**) with aryloxyacetic acid chlorides **12** and **13** (prepared from **7** & **10**) afforded the corresponding N-acyl-imidazolidinone derivatives **14** and **15** (Scheme 3).



11

12 R^1 = H, R^2 = (*E*)-propenyl
13 R^1 = OMe, R^2 = allyl

14 R^1 = H, R^2 = (*E*)-propenyl; 92%
15 R^1 = OMe, R^2 = allyl; 92%

16 R^3 = H
17 R^3 = OMe

18 R^1 = H, R^2 = (*E*)-propenyl, R^3 = H; 86%
19 R^1 = H, R^2 = (*E*)-propenyl, R^3 = OMe; 87%
20 R^1 = OMe, R^2 = allyl, R^3 = OMe; 87%

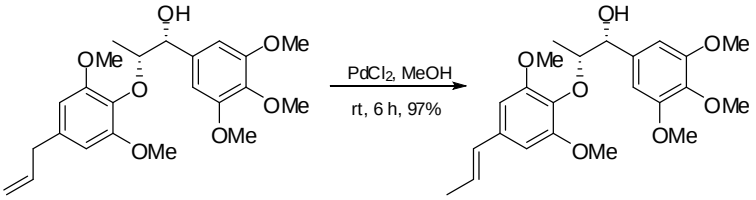
21 R^1 = H, R^2 = (*E*)-propenyl, R^3 = H; 97%
22 R^1 = H, R^2 = (*E*)-propenyl, R^3 = OMe; 98%
23 R^1 = OMe, R^2 = allyl, R^3 = OMe; 96%

1 R^1 = H, R^2 = (*E*)-propenyl, R^3 = H; 98%
2 R^1 = H, R^2 = (*E*)-propenyl, R^3 = OMe; 98%
3 R^1 = OMe, R^2 = allyl, R^3 = OMe; 98%

Scheme 3: Synthesis of (–)-Virolin (**1**), (–)-Surinamensin (**2**), (–)-Raphidecursinol B (**3**)

The aldol reaction of the boron enolates, generated from imidazolinones **14** and **15** by treating with di-*n*-butyl boron triflate in presence of Hünig's base (*i*-Pr_2NEt) with aldehyde afforded only a single diastereomer (>99% from ^{1}H-NMR of the crude

product). The reductive removal of the auxiliary from the adducts (**18**, **19** & **20**) gave the corresponding diols (**21**, **22** & **23**). Deoxygenation of primary hydroxyl group from the diols by the tosylation followed by the reductive removal of the tosylate moiety afforded (–)-virolin (**1**), (–)-surinamensin (**2**) and (–)-raphidecursinol B (**3**) (Scheme 3).



(–) - Raphidecursinol B (**3**) (–) - Polysphorin (**4**)

Scheme 4: Preparation of (–)-polysphorin (**4**)

Finally, the synthesis of (–)-polysphorin (**4**) was achieved by the isomerization of the double bond in (–)-raphidecursinol B (**3**) (Scheme 4). The spectral characteristics of the natural products prepared compare favorably with the literature reported spectral data. Structural integrity of **1**, **2**, **3** and **4** was further confirmed by the specific rotation analysis, which matched well with literature reported values. Employing this protocol, the overall yields achieved for the final compounds (–)-virolin (**1**), (–)-surinamensin (**2**), (–)-raphidecursinol B (**3**) and (–)-polysphorin (**4**) are 70%, 75%, 67% and 65% respectively starting from **5** and **8**.

Thus our study resulted in efficient and concise asymmetric total synthesis of biologically important plant metabolites (–)-virolin, (–)-surinamensin, (–)-raphidecursinol B and (–)-polysphorin has been described using two common intermediates, beginning from readily available starting materials. Since these compounds exhibit useful biological properties, present strategy facilitates the synthesis of non-natural congeners of the natural compounds with better biological profiles.

Part-B: ***Deals with the synthesis of various heterocyclic compounds and their biological evaluation, and is divided into Chapter-III and Chapter-IV.***

Chapter-III: Synthesis and Selective Cytotoxic Activity of Novel Hybrid Chalcones against Prostate Cancer (PC-3) Cells

This chapter deals with the introduction to natural and synthetic bioactive chalcones and their reported structural modifications for the development of new drug candidates. It also describes our design strategy, synthetic execution and biological evaluation of new hybrid chalcones.

Design and synthesis of new types of pharmacologically interesting hybrid chalcone analogs for drug discovery have gained much attention during recent years. Several structural modifications to the chalcone template, particularly replacement of either A or B-phenyl ring or both with heterocyclic groups have been shown to enhance their biological profiles. Even though dihydrofuro- and pyrano-chalcones are also available in nature along with their prenyl- counterparts with good biological properties, less attention was drawn towards these classes of compounds. In nature, the dihydrofuro and -pyrano fused chalcones are believed to be formed by oxidative cyclization of the prenyl analogs. The nature prefers six membered pyrano fused structures to five membered furo- fused ones as exemplified by the relatively high abundance of natural pyrano- fused chalcones. The less abundant furo fused chalcone derivatives have been reported to possess antioxidant and antimicrobial properties in addition to being chemo-preventive and cytotoxic. Against this backdrop, we designed and synthesized a new class of chalcones with dihydrobenzofuran moiety as A-ring and either substituted phenyl or pyrazole moiety as B-ring and evaluated their cytotoxic activity (fig 1).

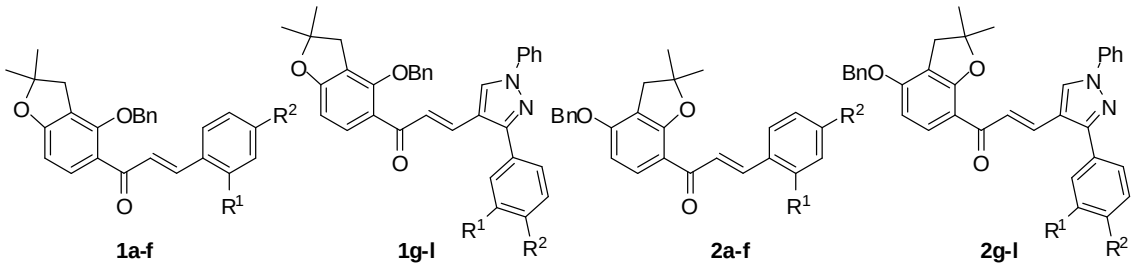
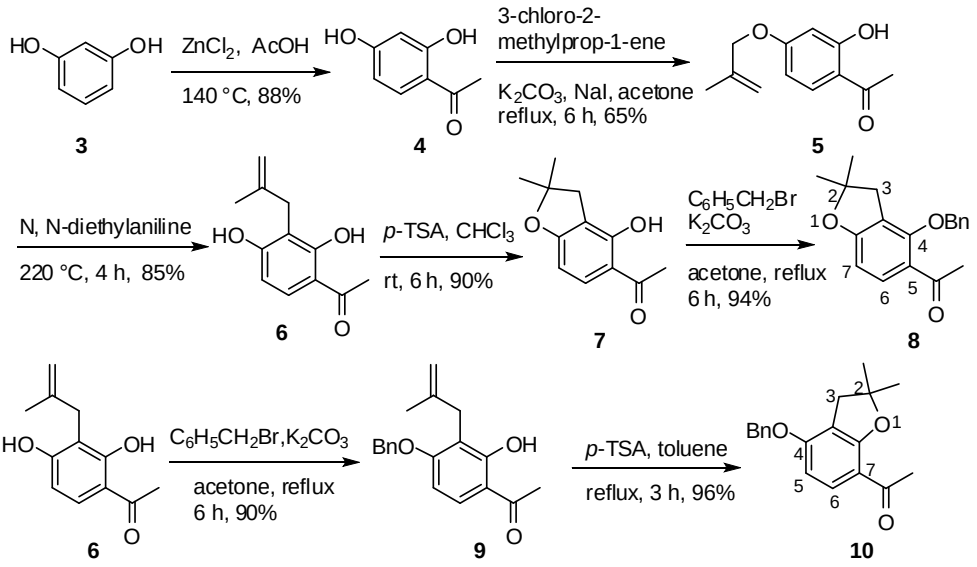


Figure 1: Chemical sructures of new hybrid chalcones **1a-l** and **2a-l**

The new class of hybrid chalcones was prepared through the ethanones **8** and **10**. Synthesis of the ethanones **8** and **10** was started from resacetophenone **4** which could be

accessed easily from resorcinol (**3**) (Scheme 1). Mono-allylation of **4** with 3-chloro-2-methylpropene gave the allyl-aryl ether **5** which on Claisen rearrangement afforded **6** in good yield. Acid catalyzed cyclization of the rearranged product **6** followed by the benzylation of the resulting benzofuran **7** afforded the ethanone **8** in very good yield. Compound **10**, the regioisomer of **8**, was prepared by benzylation of the 4-hydroxy group of **6** followed by acid catalyzed cyclization.

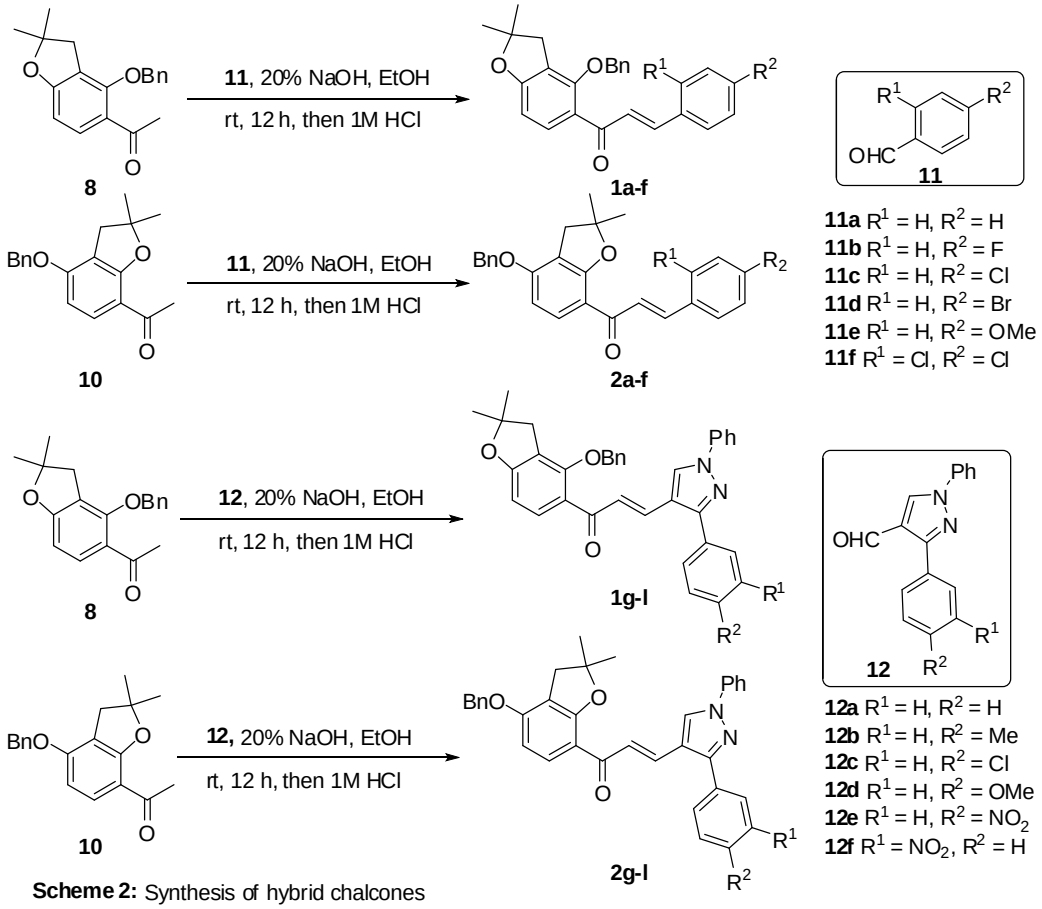


Scheme 1: Synthesis of ethanones **8** and **10**

The target 1,3-disubstituted-2-propen-1-ones (**1a-l** and **2a-l**) were prepared by the Claisen-Schmidt condensation of ethanone **8** or **10** withs various benzaldehydes (**11a-f**) and pyrazolaldehydes (**12a-f**) in ethanolic NaOH (Scheme 2). The cytotoxic potential of all newly synthesized hybrid chalcones was evaluated *in vitro* against a panel of four tumor cell lines prostate cancer (PC-3), colon cancer (HT-29), lung cancer (NCI-H460) and mouse macrophages (B-16) using MTT assay based on mitochondrial reduction of yellow MTT tetrazolium dye to a highly colored blue formazan product. Doxorubicin was used as the reference drug.

The new class of compounds showed significant to moderate cytotoxic activity on PC-3 cell lines (IC_{50} = 5.9-50 μM). Among the two regioisomeric core structures **8** and **10**, the derivatives of **10** showed more significant cytotoxic activity. It is also worthy to note that, the derivatives with pyrazole moiety as B-ring exhibited better activity over the

phenyl counterparts. Derivatives **2i**, **2j** and **2l** exhibited significantly higher cytotoxicity with IC_{50} values 8.4, 7.9 and 5.9 μM respectively and most notably showed very low or no activity on the other three cell lines.



Scheme 2: Synthesis of hybrid chalcones

In order to evaluate the effect of phenolic benzyl moiety, which initially was introduced as a protecting group to enable the synthesis, we prepared the *O*-debenzyl analogs of a few selected compounds with better activity and tested for their cytotoxic activity against PC-3 cell lines. The debenzylated compounds found to be less potent compared to the corresponding benzylated counterparts, presumably due to their decreased hydrophobicity.

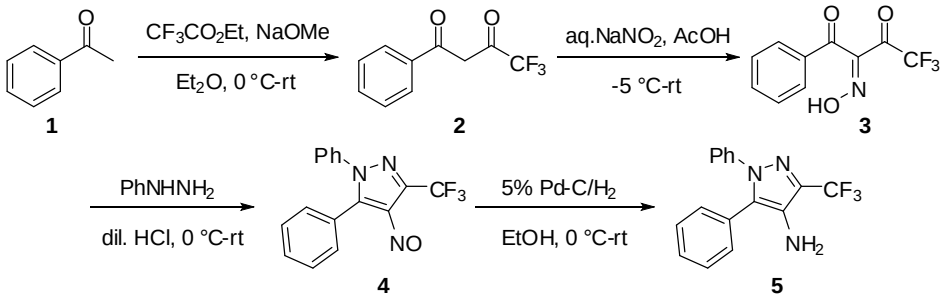
In conclusion, the present chapter described the synthesis and cytotoxic activity of a new class of hybrid-chalcones against four cell lines (PC-3, HT-29, B-16 and NCI-H460). Most of the synthesized compounds are found to be selective towards PC-3 cell line. As the selectivity of the drug is an important parameter in cancer chemotherapy, the

newly synthesized compounds particularly **2i**, **2j** and **2l** (IC_{50} = 8.4, 7.9 and 5.9 μM) could be potential anticancer drug candidates after further structure optimization.

Chapter-IV: Synthesis and Biological Evaluation of New Rhodanine-3-acetamide Derivatives

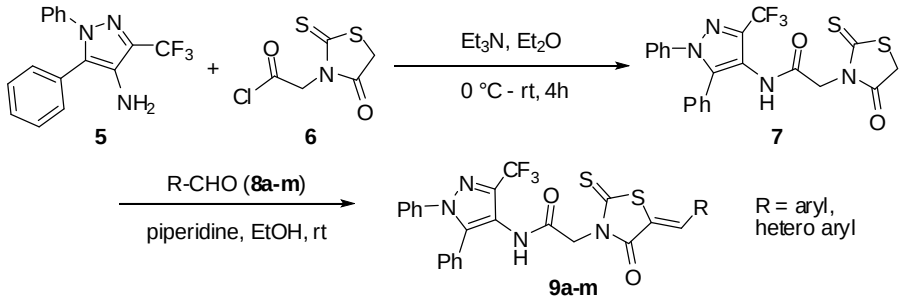
This chapter deals with the introduction to biological importance of pyrazole amines with trifluoromethyl substitution, rhodanine-3-aceticetamide derivatives and describes the design, synthesis and biological (Cytotoxic & Larvicidal) evaluation of new pyrazolyl rhodanine acetamide derivatives.

Synthesis and bioevaluation of rhodanine and rhodanine-3-acetic acid derivatives were studied extensively by many research groups. Very few reports on the synthesis and biological utility of rhodanine-3-acetamide derivatives are documented in the literature, in spite of their attractive biological profiles. Combination of two active pharmacophores within a single molecule is one of the strategies more often employed in the search for the lead molecule. The resulted new molecule would enhance the activity by an additive or synergistic effect. Inspired by this and the biological profiles of both 4-amino-3-trifluoro-pyrazoles and rhodanine-3-acetic acid derivatives reported in the literature, we reasoned that incorporation of the structural characteristics of both these moieties to result a new rhodanine-3-acetamide derivatives with newer and/or better biological profiles. In the present study the design and synthesis of new rhodanine-3-acetamide derivatives and their biological evaluation have been carried out.



Scheme 1: Synthesis of 4-amino-1,5-diphenyl-3-trifluoromethyl-pyrazole (**5**).

Synthesis of 1,5-diphenyl-3-(trifluoromethyl)-1H-pyrazol-4-amine (**5**) was started from 1,3-diketone (**2**) (Scheme 1), which in turn was prepared from acetophenone (**1**) and ethyl trifluoromethyl acetate in presence of sodium methoxide in dry ether. Treatment of 1,3-diketone (**2**) with aqueous $NaNO_2$ in acetic acid gave the diketo oxime (**3**) in good yield. Reaction of diketo oxime (**3**) with phenylhydrazine in dilute HCl afforded the nitroso-pyrazole (**4**) as a green solid. Reduction of the nitroso group by hydrogenation in presence of 10% Pd-C gave the aminopyrazole (**5**).



Scheme 2: Synthesis of pyrazolyl rhodanine acetamide derivatives (**9a-m**)

Treatment of the 4-aminopyrazole (**5**) with rhodanine-3-acetylchloride (**6**) gave the corresponding amide (**7**) in good yield. The acid chloride (**6**) was prepared from the corresponding rhodanine-3-acetic acid which in turn was prepared from glycine, carbon disulphide and sodium salt of chloroacetic acid using a reported procedure. Treatment of rhodanine-3-acetamide (**7**) with different aldehydes (**8a-m**) in presence of catalytic amount of piperidine in ethanol at room temperature gave the methylidine derivatives (**9a-m**) in good yields (Scheme 2).

The cytotoxic potential of all the newly synthesized rhodanine-3-acetamide conjugates were evaluated *in vitro* against prostate (PC-3 & DU-145) and breast (MCF-7 & MDA-MB-231) cancer cell lines using MTT assay based on mitochondrial reduction of yellow MTT tetrazolium dye to a highly colored blue formazan product. Doxorubicin was used as the reference drug. Most compounds, the heterocyclic conjugates in particular, displayed cytotoxicity against prostate cancer cell lines. The parent pyrazole amine **7** exhibited appreciable activity against breast cancer cell lines. Compound **9e** is potent against prostate cancer cell lines. The pyrazole conjugate **9k** showed substantial

cytotoxicity towards all four cell lines. The derivatives **9e** and **9k** are the most promising with IC_{50} values 12.9 & 12.5 μM against PC-3 cell line.

The larval toxicity study of all newly synthesized compounds was performed using standard protocol as described by World Health Organization (WHO, 1963), on 4th instar mosquito, *C. quinquefasciatus* larvae. The LC_{50} values of newly synthesized compounds (**5**, **7**, **9a-m**) were determined. Among these, only compound **9i** showed toxicity against *C. quinquefasciatus* (LC_{50} = 8.42 ppm, 24 h). All other derivatives including **5** and **7** showed very low / no toxicity after 24 h and 48 h.

In summary a new class of rhodanine-3-acetamide conjugates were synthesized and evaluated for their larvicidal and cytotoxic properties. Compound **9i** showed toxicity against 4th instar mosquito, *C. quinquefasciatus* larvae. The cytotoxic activity of these new derivatives were tested against prostate (PC-3 & DU-145) and breast (MCF-7 & MDA-MB-231) cancer cell lines. The derivatives **9e** and **9k** are the most promising compounds with IC_{50} values 12.9 & 12.5 μM against PC-3 cell lines.